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Detection of *Neisseria gonorrhoeae* Isolates from Tonsils and Posterior Oropharynx

M. Bissessor,[a,b] D. M. Whiley,[c,d] D. M. Lee,[a] A. F. Snow,[a] C. K. Fairley,[a,e] J. Peel,[a] C. S. Bradshaw,[a,e] J. S. Hocking,[b] M. M. Lahra,[f,g] M. Y. Chen[a,e]

Melbourne Sexual Health Centre, Melbourne, Victoria, Australia[a]; Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Victoria, Australia[b]; The University of Queensland, St. Lucia, Queensland, Australia[c]; Queensland Paediatric Infectious Diseases Laboratory, Queensland Children's Medical Research Institute, The University of Queensland, St. Lucia, Queensland, Australia[d]; Central Clinical School, Monash University, Melbourne, Victoria, Australia[e]; WHO Collaborating Centre for Sexually Transmitted Diseases, SEALS Microbiology, The Prince of Wales Hospital, Randwick, New South Wales, Australia[f]; University of New South Wales, Kensington, New South Wales, Australia[g]

We examined the factors influencing gonorrhea detection at the pharynx. One hundred men infected with *Neisseria gonorrhoeae* were swabbed from the tonsils and posterior oropharynx. *N. gonorrhoeae* was reisolated from the tonsils and posterior oropharynx in 62% and 52%, respectively ($P = 0.041$). Culture positivity was greater with higher gonococcal DNA loads at the tonsils ($P = 0.001$) and oropharynx ($P < 0.001$). *N. gonorrhoeae* can be cultured from the tonsils and posterior oropharynx with greater isolation rates where gonococcal loads are higher.

Rising gonorrhea rates, growing antimicrobial resistance, and increasingly limited antimicrobial options globally have made gonorrhea an urgent antibiotic-resistant threat (1). Pharyngeal gonorrhea is believed to be an important factor in the development of *Neisseria gonorrhoeae* antimicrobial resistance by facilitating the transfer of genetic elements of antibiotic resistance from commensal *Neisseriae*, prevalent in the pharynx, to *N. gonorrhoeae* (2).

Regular screening for pharyngeal gonorrhea is recommended for men who have sex with men (MSM). There are few published data, however, that indicate where gonococci reside within the pharynx and whether swabs should be taken from the tonsils, posterior oropharynx, or both. The primary aim of this study was to establish the specific anatomical sites from which pharyngeal gonorrhea can be detected, comparing culture and nucleic acid amplification testing (NAAT). The secondary aim was to determine the factors that influence detection using these methods, including sampling and load of *N. gonorrhoeae*.

This was a secondary study embedded within a larger study that was undertaken at the Melbourne Sexual Health Centre, Australia, as previously detailed elsewhere (3). Briefly, MSM were screened for pharyngeal gonorrhea using culture. Men who were culture positive for pharyngeal *N. gonorrhoeae* and who had not already been treated were recalled 7 days later, reswabbed from the pharynx, and retested for gonorrhea using culture and NAAT prior to treatment. First, one swab was taken from the posterior oropharynx (henceforth referred to as oropharynx), which involved sweeping the tip of a cotton swab across the posterior oropharynx without making contact with the tonsils. Then, a second swab was taken from both palatine tonsils (henceforth referred to as tonsils) where the tip of a cotton swab was swept across each tonsil, avoiding contact with the oropharynx (4, 5). All study swabs obtained from the tonsils and oropharynx were collected carefully by three sexual health clinicians (M. Bissessor, A. F. Snow, and D. M. Lee) trained in a standardized method to ensure consistent sampling.

The oropharyngeal and tonsillar swabs were plated onto separate plates for gonorrhea culture. Culture was undertaken using modified Thayer Martin medium. After inoculating culture plates, swabs were placed into 10-ml tubes containing phosphate-buffered solution for NAAT detection (6).

Detection of *N. gonorrhoeae* DNA in samples was performed using individual real-time PCR methods targeting the gonococcal *porA* pseudogene and multicopy *opa* genes (*porA* monoplex and *opa* monoplex) as previously described (6). An NAAT result was reported as positive when the two assays were positive. The cycle threshold (C_T) values obtained using the *porA* monoplex and *opa* monoplex real-time PCR were used as semiquantitative measures of gonococcal DNA. C_T values are inversely proportional to the gonococcal load; that is, the lower the C_T value, the higher the gonococcal load. A difference of 3.3 cycles is generally considered to represent a 10-fold difference in DNA load (7).

To assess sampling adequacy, DNA extracts were tested using a real-time PCR assay targeting human endogenous retrovirus 3 (ERV3) as previously described (8). ERV3 real-time PCR values were used as semiquantitative measures of human DNA.

The calculation of the sample size has been previously described for the larger study (5). The chi-square test was used to compare categorical data, and McNemar's test was used to compare paired proportions. The Mann-Whitney U test was used to compare organism loads between groups. Ethical approval for this study was granted by the Alfred Hospital Research Ethics Committee (no. 453/11).

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Address correspondence to M. Bissessor, mbissessor@mshc.org.au.

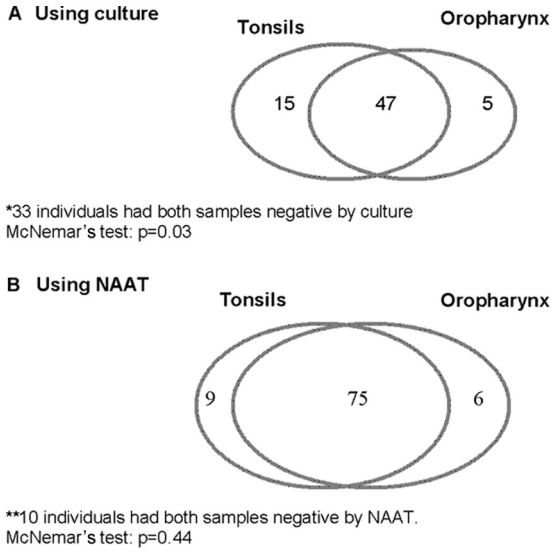

FIG 1 Number of positive gonorrhea swabs among 100 men with culture-positive pharyngeal gonorrhea according to site of sampling.

One hundred men with pharyngeal gonorrhea were recruited into the study. The median time between the diagnosis of pharyngeal gonorrhea and retesting and treatment was 7 days (range, 6 to 9 days). The demographic and behavioral characteristics of men and pharyngeal isolate MICs have been detailed elsewhere (3).

N. gonorrhoeae was cultured from the tonsils as well as the oropharynx (62% versus 52%, respectively; $P = 0.041$). In 47 men, the culture was positive at the tonsils and the oropharynx. There were 15 men whose culture was positive at the tonsils but negative at the oropharynx, and there were 5 men whose culture was positive at the oropharynx but negative at the tonsils (Fig. 1A). A further 33 men were culture negative at the two sites.

N. gonorrhoeae was detected using NAAT in 84% of tonsillar and 81% of oropharyngeal samples. These results are summarized Table 1. NAAT for *N. gonorrhoeae* isolates from the tonsils and oropharynx was significantly more sensitive than culture at the tonsils (84% versus 62%; $P < 0.001$) and the oropharynx (81% versus 52%; $P < 0.001$). There was no significant difference in detection between the tonsils and oropharynx using NAAT ($P = 0.61$). In 75 men, NAAT was positive at the tonsils and oropharynx. In 9 men, NAAT was positive at the tonsils but negative at the oropharynx, and in 6 men, NAAT was positive at the oropharynx but negative at the tonsils (Fig. 1B). A further 10 men were NAAT negative at the two sites.

Analysis of ERV3 C_T values indicated that detection of gonorrhea was influenced by the adequacy of sampling (Table 1). At the oropharynx, the median ERV3 C_T value was significantly lower (indicating higher human DNA load) in culture-positive men than that in culture-negative men (26.8 cycles versus 27.8 cycles; $P = 0.013$). At the tonsil, the median ERV3 C_T value was not significantly lower in culture-positive men than that in culture-negative men (26.3 cycles versus 27.0 cycles; $P = 0.182$). Sampling also influenced gonorrhea detection by NAAT. At the tonsils, the median ERV3 C_T value was significantly lower in NAAT-positive men than that in NAAT-negative men (26.2 cycles versus 27.3; $P = 0.040$). Similarly, at the oropharynx, the median ERV3 C_T value was significantly lower in NAAT-positive men than that in NAAT-negative men (26.9 cycles versus 27.5; $P = 0.045$). However, in all of these observations, the difference in ERV3 C_T values corresponded to a 10-fold difference in DNA load.

At the tonsils, the median *opa* monoplex real-time PCR C_T values were significantly lower (indicating higher gonococcal DNA load) in culture-positive specimens than those in culture-negative specimens (28.0 cycles and 31.4 cycles; $P = 0.001$). Similarly, at the oropharynx, the median C_T values of *opa* monoplex real-time PCR were significantly lower in culture-positive specimens than those in culture-negative specimens (28.5 cycles and 33 cycles; $P < 0.001$). In the above observations, the difference in C_T values corresponded to a >10-fold difference in DNA load.

In this study, we determined the sites within the pharynx from which *N. gonorrhoeae* can be detected using two different laboratory methods. Using culture, *N. gonorrhoeae* was isolated from the tonsils and oropharynx separately. NAAT also detected *N. gonorrhoeae* separately from the tonsils and oropharynx but with significantly greater sensitivity. The isolation rate from culture was significantly higher with higher gonococcal load and better sampling. Detection using NAAT was also improved with better sampling.

We included culture in this study because isolation of *N. gonorrhoeae* requires a live organism and is, therefore, indicative of productive infection. While pharyngeal screening for gonorrhea

TABLE 1 Frequency of results for differing combinations of *N. gonorrhoeae* culture, real-time PCR, and ERV3 real-time PCR

No. of men	Tonsils[a]			Oropharynx[a]		
	Culture	*N. gonorrhoeae* PCR (C_T [range; mean])	ERV3 PCR (C_T [range; mean])	Culture	*N. gonorrhoeae* PCR (C_T [range; mean])	ERV3 PCR (C_T [range; mean])
45	+	+ (23–36; 29)	+ (22–29; 26)	+	+ (22–35; 28)	+ (23–30; 26)
2	+	−	+ (26–26; 26)	+	+ (33–37; 36)	+ (28–28; 28)
12	+	+ (26–31; 28)	+ (24–28; 26)	−	+ (25–35; 30)	+ (24–30; 27)
2	+	+ (35–37; 36)	+ (27–29; 28)	−	−	+ (29–29; 29)
1	+	−	+ (29)	−	+ (35)	+ (29)
4	−	+ (31–32; 31)	+ (24–27; 26)	+	+ (29–30; 29)	+ (25–29; 26)
1	−	−	+ (29)	+	+ (33)	+ (27)
14	−	+ (24–36; 31)	+ (24–31; 27)	−	+ (24–37; 32)	+ (24–29; 27)
7	−	+ (32–38; 35)	+ (23–31; 27)	−	−	+ (26–32; 28)
2	−	−	+ (27–29; 28)	−	+ (35–38; 36)	+ (28–28; 28)
10	−	−	+ (24–30; 27)	−	−	+ (25–30; 27)

[a] +, positive; −, negative.

using NAAT has increased in clinical practice, culture remains important for the determination of antimicrobial susceptibility and test of cure following treatment for gonorrhea (3).

Adequate sampling is important for optimal detection of pharyngeal gonorrhea using culture (4, 5). We have also shown that sampling adequacy is important for the detection of *N. gonorrhoeae* at the tonsils and the oropharynx using NAAT even though NAAT is a more sensitive method for detecting *N. gonorrhoeae*. In addition, even when NAAT testing is used, it is important to sample the oropharynx and tonsils.

Previously, we showed that lower-load gonococcal infections in the pharynx were more likely to be missed using culture (9). In this study, we found that the failure of culture to detect lower-load gonococcal infections applied to the tonsils and the posterior oropharynx independently.

Although the positivity from culture taken from the tonsils was significantly higher than that from the oropharynx, both were low considering that all men in the study were culture-positive from screening that was undertaken a median of 7 days prior. Previous studies using culture suggest that pharyngeal gonorrhea is generally transient (10, 11) and self-limiting over a period of weeks or months. It would, therefore, seem unlikely that spontaneous clearance of infection would account for the poor yield when each site was sampled independently within 7 days. Rather, it may reflect the fact that optimal yield using culture requires swabbing from the two sites to obtain sufficient bacterial load to support culture. Ostensibly, this would also aid in detection of pharyngeal *N. gonorrhoeae* by NAAT.

There are strengths and limitations to this study. Culture for gonorrhea requires stringent transport and laboratory conditions. The on-site location of our laboratory with the ability to culture at the point of collection, as well as the rigorous laboratory methods, ensured optimal conditions for *N. gonorrhoeae* growth. A limitation is that although there was a high level of emphasis placed on careful sampling from the tonsils and oropharynx, there was still some variability in sampling as indicated by the analyses of ERV3.

Previous studies have investigated the sensitivity of oral throat rinses for the detection of pharyngeal gonorrhea. These are potentially more acceptable, as swabs may induce a gag reflex making collection uncomfortable for patients and impeding proper sampling. However, in a study of 25 cases of pharyngeal gonorrhea, the sensitivity of NAAT on oral-rinse specimens was only 72% (12).

The asymptomatic nature of pharyngeal gonorrhea, its propensity for selection of antimicrobial-resistant clones, and treatment failures underscore the importance of optimal detection of pharyngeal gonorrhea and verification of cure.

This study has established that *N. gonorrhoeae* can be isolated from the tonsils and the oropharynx. Clinicians screening for pharyngeal gonorrhea using culture or NAAT should be aware of the higher sensitivity of NAAT over that of culture and should be trained to ensure that one swab is collected carefully and thoroughly from each of these sites for optimal detection of *N. gonorrhoeae*, regardless of the laboratory method used.

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We declare that we have no conflicts of interest.

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